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Adverse Effects of Intraportal Chemotherapy on Natural Killer Cell Activity in Colorectal Cancer Patients

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Background: Adjuvant intraportal chemotherapy has been used with a view to prevent the development of metachronous hepatic metastases following curative resection for colorectal cancer. To evaluate the effects of this therapy on systemic antitumor immunological activity, 35 colorectal cancer patients who underwent curative resection were investigated.
Method: Among them, 19 had adjuvant intraportal chemotherapy with mitomycin C and 5-fluorouracil (treated group) and 16 had no chemotherapy (untreated group). Natural killer (NK) cell activity, lymphocyte subpopulations, and immunosuppressive acidic protein (IAP) in the peripheral blood were measured serially before and after operation, and the values were compared between the two groups.
Result: The NK cell activity and the percentages of CD16 positive and CD56 positive cells were markedly reduced in the treated group postoperatively. Significant difference was also observed between the two groups on the 4th postoperative day in regard to NK cell activity and CD56 positive cells.
Conclusions: Intraportal chemotherapy in our study reduced the NK cell activity and its population in the peripheral blood.
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KEY WORDS: hepatic metastases; 5-fluorouracil; mitomycin C; natural killer cell activity; T-cell subpopulations; immunosuppressive acidic protein (IAP)

INTRODUCTION

The liver is a favorable site for distant metastases in colorectal cancer, and once they become established, the prognosis is bleak [1]. Metachronous hepatic metastases may develop either from undetectable micrometastases that may already have been established or from the intraportal migration of malignant cells, brought about by the manipulation of the primary tumor at surgery. In an attempt to reduce the subsequent development of hepatic metastases in putatively curable colorectal cancer, adjuvant intraportal chemotherapy was advocated [2] following the demonstration of tumor cells in the venous drainage of resected specimens of carcinoma of the colon [3] and the effectiveness of intraportal administration of nitrogen mustard on the day of injection of cancer cells into the portal vein by significant reduction of liver tumor ''takes'' in rats [4]. Until now, several randomized prospective clinical trials assessing the value of perioperative portal-vein infusion of single [5-fluorouracil (5-FU)] [5–8] or combination [5-FU and mitomycin C (MMC)] [9,10] of chemotherapeutic agents have been carried out. The results are, however, at best equivocal in preventing hepatic metastases. Accordingly, this therapy is yet to be recommended outside the clinical trials.

Although several randomized clinical trials have been carried out, antitumor immunological activity of the host during intraportal chemotherapy has not yet been studied in depth. In our previous study, we evaluated the effects

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of intraportal administration of chemotherapeutic agents on natural killer (NK) cell activity in the rat liver. Hepatic NK cell activity was found to be reduced with the administration of chemotherapeutic agents (MMC and 5-FU) [11].

In this study, we investigated the effects of perioperative intraportal chemotherapy on the systemic antitumor immunological activity in the patients with colorectal cancer by estimating repeatedly the NK cell activity, lymphocyte subpopulations, and immunosuppressive acidic protein (IAP) in the peripheral blood.

MATERIALS AND METHODS

Patients

Between 1992 and 1994, 35 colorectal cancer patients who underwent macroscopic curative resection in Shinshu University Hospital were subjected in this study. No complications were found in the postoperative course in these patients. Among the 35 patients, 19 had adjuvant intraportal chemotherapy (treated group) and 16 had no chemotherapy (untreated group). In our institution, the intraportal chemotherapy was performed for the patients with advanced but putatively resectable colorectal cancer and was not performed for early cancer. We did not perform this therapy for the patients who had serious co-existent disease, or for the patients who had undergone upper abdominal surgery.

Clinicopathological backgrounds of the patients are shown in Table I. No significant differences were observed with respect to age, sex, location of tumor, operative procedure, co-existence of other diseases, and postoperative recurrences between the two groups. A significant difference, however, was observed in Dukes' stage of the disease. The incidence of Dukes' A stage was significantly higher in the untreated group ($P < 0.05$; χ^2 test).

TABLE I. Effect of Intraportal Chemotherapy on Natural Killer Cell Activity in Colorectal Cancer: Comparison of Clinicopathological Features of Treated and Untreated Groups

	Treated group (n = 19)	Untreated group (n = 16)	Significance
Age	67.5 ± 8.1	68.0 ± 12.0	NS
Gender			
Male	11	9	NS
Female	8	7	
Location of tumor			
Colon	10	9	NS
Rectum	9	7	
Operative procedure			
Colectomy	10	9	
Low anterior resection	8	6	NS
Miles operation	1	1	
Dukes' stage of the disease			
A	2	8	$P < 0.05$
B	11	5	NS
C	6	3	
Co-existent diseases			
Cardiovascular	1	4	
Hepatic	0	2	NS
Pulmonary	2	0	
Cerebral	1	0	
Postoperative recurrences			
None	15	13	
Hepatic	1	2	NS
Nodal	2	1	
Peritoneal	1	0	

NS = not significant.

Intraportal Chemotherapy

At the time of radical operation for colorectal cancer, we inserted an intravenous hyperalimentation catheter (Nipro, Osaka, Japan) for ~10 cm through the right gastroepiploic vein toward portal vein immediately after laparotomy. Soon after, 10 mg of MMC (Kyowa Hakko, Tokyo, Japan) in 200 ml of normal saline was infused through the catheter at 50 ml/hr by an infusion pump. Following the infusion of MMC, 500 mg of 5-FU (Kyowa Hakko) in 500 ml of normal saline was infused through the catheter at 500 ml/day by an infusion pump. After the continuous infusion of 5-FU for 7 days, intraportal chemotherapy was stopped and the catheter was pulled out. We did not administer any other anticancer agents until the 14th postoperative day (POD). In the untreated group, no anticancer agents were administered at preoperative, intraoperative, or postoperative period. Blood samples were drawn from the peripheral vein on preoperative, and 1st, 4th, 7th, and 14th POD.

Radioisotope angiography after administration of ^{99m}Tc through the intraportal catheter shows diffuse distribution of the agents in the liver, presented in Figure 1.

Measurement of NK Cell Activity

Mononuclear cells were separated from heparinized blood on a Ficoll-Hypaque (density, 1.077 g/ml; Lymphoprep, Nycomed Pharma As, Oslo, Norway) density gradient. NK cell activity was measured by a standard 4-hour ^{51}Cr-release assay, using K 562 as target cells at effector/target cell (E/T) ratios of 40:1 and 20:1. NK cell activity, expressed as % cytotoxicity, was calculated by the following formula: % cytotoxicity = [(experimental release − spontaneous release)/(total release - spontaneous release)] × 100.

Flow Cytometric Analysis of Lymphocyte Subpopulations

Mononuclear cells were separated from heparinized blood on a Ficoll-Hypaque density gradient. Cells were cryopreserved by a mechanical freezing method [12] and stored in the liquid nitrogen tank until all specimens from each patient could be tested at the same time. After thaw-

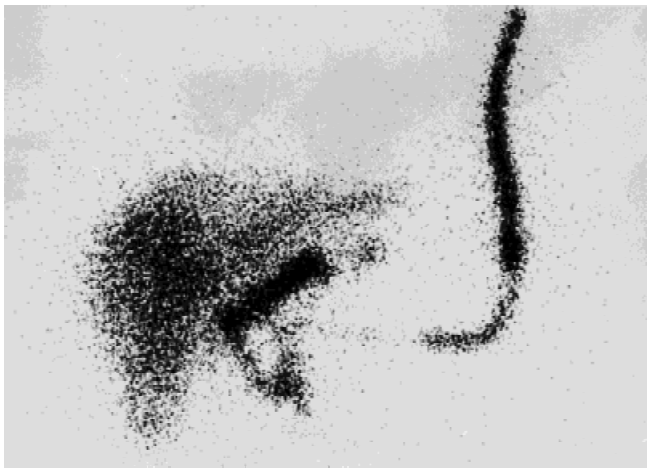

Fig. 1. Radioisotope angiography after administration of ^{99m}Tc through the intraportal catheter. The agents were diffusely distributed into the liver 12 seconds after the administration.

ing the cells in a 37°C waterbath, they were washed with cold RPMI-1640 medium supplemented with 20% heat-inactivated fetal calf serum. Cell viability tested by trypan blue exclusion exceeded 95% both before and after cryopreservation.

Cells were incubated for 30 minutes with fluorescein isothiocyanate conjugated anti-Leu-3a (CD4), anti-TCR-α/β-1, and anti-Leu-11a (CD16), phycoerythrin-conjugated anti-Leu-2a (CD8), anti-TCR-γ/δ, and anti-Leu-19 (CD56), peridinin chlorophyll protein conjugated anti-HLA-DR and CD3 (Leu™-4) monoclonal antibodies (Becton Dickinson Immunocytometry System, San Jose, CA), and three-color analysis were performed with a FACScan™ (Becton Dickinson, Mountain View, CA). Lymphocytes were gated and the percentages of stained cells were calculated by counting 20,000 cells in each experiment.

Measurement of Immunosuppressive Acidic Protein (IAP) in Peripheral Blood

Serum was separated and stored at −20°C until use. After thawing, IAP was measured by turbidimetric immunoassay using SANTEST IAP-N kit (Sanko Junyaku, Tokyo, Japan) with an autoanalyser (Hitachi 7150).

Statistical Analysis

Values were expressed as means ± SD. In each group, two-way analysis of variance (ANOVA) was used with NK cell activity, percentages of lymphocyte subpopulations and serum IAP as dependent variables, and time and patients as factors. If the *P* value for the overall analysis of variance with time was significant, Scheffé's multiple comparison test was used to determine which pairwise comparisons were statistically significant. One-way ANOVA was used to compare the two groups on the same day of investigation. A value of $P < 0.05$ was considered significant.

RESULTS

By using two-way ANOVA with the immunological parameters as the dependent variables and individual patients as one of the factors, we found significant differences ($P < 0.01$) among the patients in almost all the groups set up in each category.

Comparison of NK Cell Activity in Peripheral Blood

The activity was measured in 15 patients with 8 in the treated and 7 in the untreated group. The activity with the E/T ratio of 40:1 (Fig. 2A) was significantly lower on the 1st, 4th, 7th, and 14th POD than that of the preoperative level in the treated group ($P < 0.01$, 0.01, 0.05, and 0.01, respectively). The activity with the E/T ratio of 20:1 (Fig. 2B) was also significantly lower on the 1st, 4th, and 14th POD than that of the preoperative level in the treated group ($P < 0.05$, 0.01, and 0.05, respectively). No significant changes, however, were observed in the untreated group in both the E/T ratios with time.

Comparing the NK cell activity between the treated and untreated groups on the same day of estimation, the activity on the 4th POD in the treated group was significantly lower than that of the untreated group in both E/T ratios of 40:1 [$(24.4 \pm 10.2$ vs. $35.1 \pm 5.7)$; $P < 0.05$] and 20:1 [$(16.0 \pm 5.6$ vs. $23.0 \pm 4.7)$; $P < 0.05$].

Analysis of Lymphocyte Subpopulations

Lymphocyte subpopulations of peripheral blood were analyzed in 18 patients with 9 in each group.

Lymphocytes expressing the surface marker of NK cells (CD16 positive and CD56 positive cells). The percentages of CD16 positive cells (Fig. 3A) in the treated group on the 1st, 4th, 7th, and 14th POD were significantly lower than that of the preoperative value ($P < 0.05$, 0.01, 0.01, and 0.01, respectively). In the case of CD56 positive cells (Fig. 3B), the percentages in the treated group on the 1st, 4th, 7th, and 14th POD were significantly lower than that of the preoperative value ($P < 0.05$, 0.01, 0.01, and 0.05, respectively). In the untreated group, however, no significant changes were observed with time.

Comparing CD16 positive and CD56 positive cells between the two groups on the same day of estimation, the percentage of CD56 positive cells in the treated group was significantly lower than that of the untreated group on the 4th POD (22.3 ± 11.4 vs. 32.2 ± 7.3; $P < 0.05$).

T-lymphocyte subpopulations (CD3, CD4 and CD8 positive cells). The percentage of CD3 positive cells is shown in Figure 4A. The percentage of these cells was found to be highest on the 7th POD in both the treated and untreated groups. When CD3 and TCR α/β double-positive cells and CD3 and TCR γ/δ double-positive

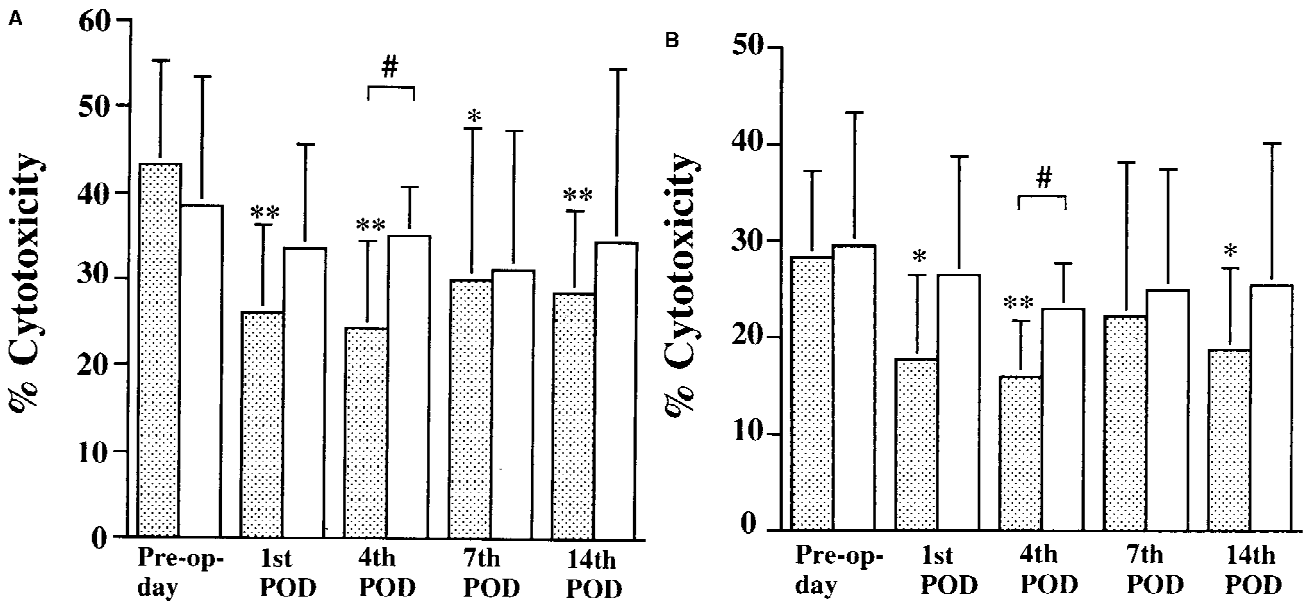

Fig. 2. Natural killer cell activity in the peripheral blood expressed as % cytotoxicity. **A.** Activity at effector/target ratio of 40:1. **B.** Activity at effector/target ratio of 20:1. Values are expressed as mean with SD as vertical lines. Stippled bar stands for the treated (n = 8) and open bar for the untreated group (n = 7). Pre-op-day: Preoperative day. POD: Postoperative day. *:$P < 0.05$, **:$P < 0.01$ (significantly different from preoperative value). ≠:$P < 0.05$, significantly different between the treated and untreated groups.

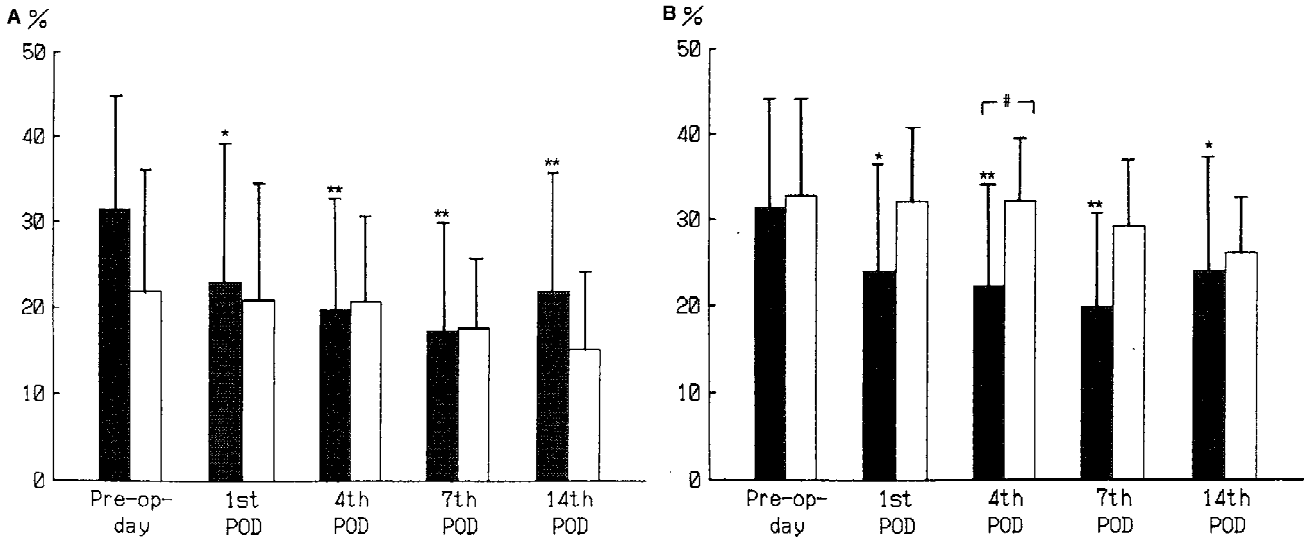

Fig. 3. The percentage of lymphocytes expressing the surface marker of natural killer cells in the peripheral blood. **A.** Percentage of CD16 positive cells. **B.** Percentage of CD56 positive cells. Values are expressed as mean with SD as vertical lines. Stippled bar stands for the treated (n = 9) and open bar for the untreated group (n = 9). Pre-op-day: preoperative day. POD: postoperative day. *:$P < 0.05$, **:$P < 0.01$ (significantly different from preoperative value). ≠:$P < 0.05$, significantly different between the treated and untreated groups.

cells were studied, the percentage of CD3 and TCR α/β double-positive cells on 7th POD in both the groups was found to be higher than the preoperative values, but the percentage of CD3 and TCR γ/δ double-positive cells did not show any significant change with time (data not shown).

The percentage of CD4 positive cells is shown in Figure 4B. The percentage of these cells reached the peak level on the 7th POD in both the groups. Although CD4 and HLA-DR double-positive cells were analyzed for further examination of CD4 positive cells, no significant differences were observed with time (data not shown).

The percentage of CD8 positive cells in the treated group decreased in the course of time, but that in the untreated group showed no change with time, as shown in Figure 4C. Although CD8 and HLA-DR double-positive cells were analyzed for further investigation, no

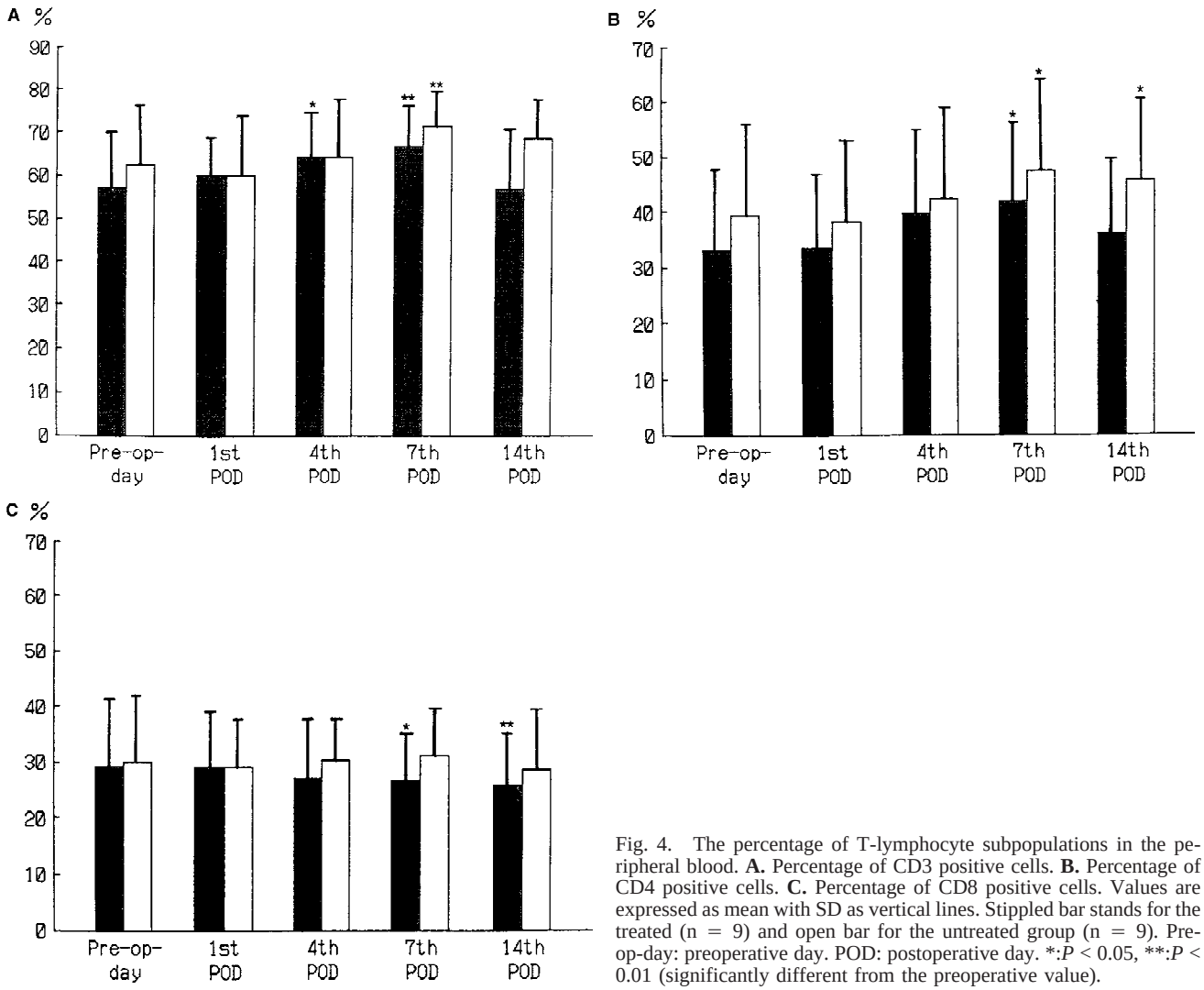

Fig. 4. The percentage of T-lymphocyte subpopulations in the peripheral blood. **A.** Percentage of CD3 positive cells. **B.** Percentage of CD4 positive cells. **C.** Percentage of CD8 positive cells. Values are expressed as mean with SD as vertical lines. Stippled bar stands for the treated (n = 9) and open bar for the untreated group (n = 9). Pre-op-day: preoperative day. POD: postoperative day. *:$P < 0.05$, **:$P < 0.01$ (significantly different from the preoperative value).

tendency of alteration with time was detected (data not shown).

Comparing the treated and untreated groups, no significant differences were observed on the same day of estimation in any of the T-cell subpopulations.

Analysis of IAP

IAP was measured in 11 patients in the treated and 9 in the untreated group, and these data are shown in Figure 5. Preoperative serum IAP levels were normal (< 500 μg/ml) in all the 20 patients. IAP levels in both the groups reached their maximum on the 4th POD and then decreased gradually. No significant differences, however, were observed between the treated and untreated groups on the same day of estimation.

DISCUSSION

In this study, immunological parameters were estimated in colorectal cancer patients treated by surgery with adjuvant intraportal chemotherapy and were compared with those in patients treated by surgery alone. As a parameter of nonspecific cellular immunity, NK cell activity and its percentages in lymphocyte population were estimated. As a parameter of specific antitumor immunity, lymphocyte subpopulations of T cells were evaluated. Furthermore, as an immunosuppressive agent in tumor bearing host, IAP in the peripheral blood was measured.

Patients' background in the treated and untreated groups was different in regard to the Dukes' stage only. In our institution, intraportal chemotherapy was performed usually for the patients with advanced but putatively resectable colorectal cancer and was not performed for early cancer. Accordingly, the incidence of Dukes' A stage was higher in the untreated than the treated group. It has been reported that the level of NK cells [13,14] and its cytotoxicity [14,15] do not have any relationship with the stages of colorectal cancer. The only time that NK cytotoxicity seems to correlate with stage is when patients have very advanced metastatic disease [15,16]. So,

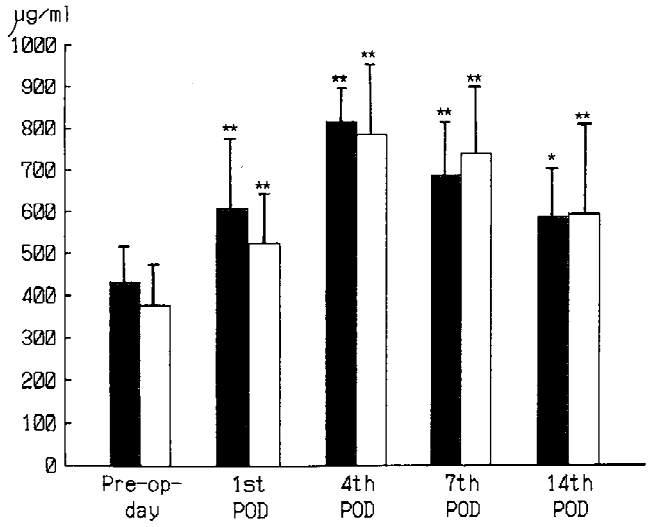

Fig. 5. Immunosuppressive acidic protein (IAP) concentrations in the peripheral blood. Values are expressed as mean with standard deviations as vertical lines. Stippled bar represents the treated (n = 11) and the clear bar the untreated group (n = 9). Pre-op-day: preoperative day; POD: postoperative day. *:$P < 0.05$, **:$P < 0.01$ (significantly different from the preoperative value).

the difference in Dukes' stage was considered to be minimal, because highly advanced cases with Dukes' D stage in which the immunological activity is thought to be altered were not included in this study.

It is reported that surgical stress and anesthesia inhibit NK cell activity in the immediate postoperative period [17,18]. Although NK cell activity of peripheral blood in our study revealed the tendency of reduction in both the groups, the treated group demonstrated marked suppression in the postoperative period. Comparing the activity between the treated and untreated groups showed significant reduction in the treated group on the 4th POD. Therefore, intraportal chemotherapy using MMC and 5-FU may suppress NK cell activity in the peripheral blood. We had reported in our animal experiment that hepatic NK cell activity is reduced by intraportal infusion of MMC for 4 hours and 5-FU for 5 days [11]. Concentrations of MMC and 5-FU used in our animal experiment were similar to those in the present study. These results indicate that intraportal chemotherapy may suppress not only hepatic, but also peripheral NK cell activity.

CD16 positive and CD56 positive cells, which represent NK cells [19], showed remarkable diminution postoperatively in the treated group. In contrast to the treated group, CD16 positive and CD56 positive cells in the untreated group showed no significant diminution. Furthermore, comparative study of CD56 positive cells between the treated and untreated groups showed significant reduction in the treated group on the 4th POD. Thus intraportal chemotherapy may reduce peripheral lymphocytes expressing the surface marker of NK cells. Since the correlation exists between the level of NK cells and its function [13], this reduction corresponds to the suppression of NK cell activity in this study.

Although slight changes in CD3 positive, CD4 positive, and CD8 positive cells, representing pan-T cells, helper/inducer T cells, and suppresser/cytotoxic T cells [19], respectively, were observed in the course of time, no significant differences were found between the treated and untreated groups. Studies on activated T cells, as CD4 and HLA-DR double-positive cells, or CD8 and HLA-DR double-positive cells [20], and CD3 related TCR α/β and TCR γ/δ, showed no differences between the two groups. These results suggest that surgery rather than intraportal chemotherapy may have some influence on various subsets of T cell population.

IAP is an immunosuppressive agent first detected in the malignant ascitic fluid and sera of cancer patients by Tamura et al. [21]. They found that IAP is released in the culture medium of normal macrophages and granulocytes. Later, Shimizu et al. [22] suggested that IAP may be produced by cancerous tissue and/or lymphocytes or macrophages in contact with cancer cells. Wherever it may originate, it is found to be increased in patients with advanced cancer [23]. Shown to inhibit the immune surveillance system [24], this protein is thought to be useful for diagnostic purposes [25] and estimating the immunological state, clinical course, and surgical curability in cancer patients [23]. Fujimoto et al. [26] reported that serum IAP levels increase in the first postoperative week and then decline gradually. In our study, serum IAP level was also increased in course of time postoperatively, but no significant differences were found between the treated and untreated groups. Therefore, surgery rather than intraportal chemotherapy influences the serum IAP level.

The findings in this study show that the intraportal chemotherapy has a suppressive effect on NK cells and may not have strong influence on T cells. NK cells which play an important role in nonspecific immunosurveillance, also inhibit the metastatic process in its early stages [27,28]. Thus it can be postulated that although intraportal chemotherapy is advocated to prevent hepatic metastases, it may also exert an unfavorable influence on immunological activity of the host, which plays an important role in preventing metastases.

Since two kinds of anticancerous agents, MMC and 5-FU, were used for intraportal chemotherapy in our study, it is difficult to explain which kind of agent suppresses NK cell activity. In an animal experiment, Ishida et al.[29] demonstrated the suppression of hepatic NK cell activity by intraportal administration of 5-FU alone. We can, therefore, assume that both the regimens (5-FU or 5-FU/MMC combination), being used in clinical trials for intraportal chemotherapy, suppress NK cell activity in the host. We have demonstrated the recovery of reduced hepatic NK cell activity with the intraportal ad-

ministration of MMC and 5-FU by adding Lentinan, a biological response modifier, with the cytotoxic agents [11].

CONCLUSIONS

It can thus be concluded that intraportal chemotherapy (MMC/5-FU) reduces the NK cell activity and its population and does not have significant influence on T-cell subpopulations or IAP in the peripheral blood. It also can be speculated that the appropriate use of biological response modifiers with the chemotherapeutic agents may improve the outcome of this therapy.

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REFERENCES

1. Taylor I, Brooman P, Rowling JT: Adjuvant liver perfusion in colorectal cancer: Initial results of a clinical trial. Br Med J 1977; 2:1320–1322.
2. Morales F, Bell M, McDonald GO, Cole WH: The prophylactic treatment of cancer at the time of operation. Ann Surg 1957; 146:588–593.
3. Fisher ER, Turnbull RB Jr: The cytologic demonstration and significance of tumor cells in the mesenteric venous blood in patients with colorectal carcinoma. Surg Gynecol Obstet 1955;100:102–108.
4. Cruz EP, McDonald GO, Cole WH: Prophylactic treatment of cancer: The use of chemotherapeutic agents to prevent tumor metastasis. Surgery 1956;40:291–296.
5. Taylor I, Machin D, Mullee M, et al.; A randomized controlled trial of adjuvant portal vein cytotoxic perfusion in colorectal cancer. Br J Surg 1985;72:359–363.
6. Fielding LP, Hittinger R, Grace RH, Fry JS: Randomised controlled trial of adjuvant chemotherapy by portal-vein perfusion after curative resection for colorectal adenocarcinoma. Lancet 1992;340:502–506.
7. Wolmark N, Rockette H, Wickerham DL, et al.: Adjuvant therapy of Dukes' A, B, and C adenocarcinoma of the colon with portal-vein fluorouracil hepatic infusion: Preliminary results of National Surgical Adjuvant Breast and Bowel Project Protocol C-02. J Clin Oncol 1990;8:1466–1475.
8. Wereldsma JCJ, Bruggink EDM, Meijer WS, et al.: Adjuvant portal liver infusion in colorectal cancer with 5-fluorouracil/heparin versus urokinase versus control: Results of a prospective randomized clinical trial (colorectal adenocarcinoma trial I). Cancer 1990;65:425–432.
9. Ryan J, Weiden P, Crowley J, Bloch K: Adjuvant portal vein infusion for colorectal cancer: A 3-arm randomized trial [abstract]. Proc Am Soc Clin Oncol 1988;7:95A.
10. Swiss Group for Clinical Cancer Research (SAKK): Long-term results of single course of adjuvant intraportal chemotherapy for colorectal cancer. Lancet 1995;345:349–353.
11. Rafique M, Adachi W: Effects of intraportal administration of chemoimmunotherapeutic agents on natural killer cell activity in the rat liver. J Surg Oncol 1995;60:154–159.
12. Ichino Y, Ishikawa T: Cytolysis of autologous fresh osteosarcoma cells by human cytotoxic T lymphocytes propagated with T cell growth factor. Gann 1983;74:584–594.
13. Balch CM, Tilden AB, Dougherty PA, et al.: Depressed levels of granular lymphocytes with natural killer (NK) cell function in 247 cancer patients. Ann Surg 1983;198:192–199.
14. Tartter PI, Martinelli G, Steinberg B, Barron D: Changes in peripheral T-cell subsets and natural-killer cytotoxicity in relation to colorectal cancer surgery. Cancer Detect Prev 1986;9:359–364.
15. Tartter PI, Steinberg B, Barron DM, Martinelli G: The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. Arch Surg 1987;122:1264–1268.
16. Pross HF, Lotzová E: Role of natural killer cells in cancer. Nat Immun 1993;12:279–292.
17. Tønnesen E, Hüttel MS, Christensen NJ, Schmitz O: Natural killer cell activity in patients undergoing upper abdominal surgery: Relationship to the endocrine stress response. Acta Anaesthesiol Scand 1984;28:654–660.
18. Tønnesen E, Wahlgreen C: Influence of extradural and general anaesthesia on natural killer cell activity and lymphocyte subpopulations in patients undergoing hysterectomy. Br J Anaesth 1988;60:500–507.
19. Samuelson J, von Lichtenberg F: Diseases of immunity. In Cotran RS, Kumar V, Robbins SL (eds): ''Robbins Pathologic Basis of Disease,'' 5th ed. Philadelphia: W.B. Saunders, 1994,171–240.
20. Tomkinson BE, Wagner DK, Nelson DL, Sullivan JL: Activated lymphocytes during acute Epstein-Barr virus infection. J Immunol 1987;139:3802–3807.
21. Tamura K, Shibata Y, Matsuda Y, Ishida N: Isolation and characterization of an immunosuppressive acidic protein from ascitic fluids of cancer patients. Cancer Res 1981;41:3244–3252.
22. Shimizu N, Yamane T, Karino T, et al.: Immunosuppressive acidic protein (IAP) in gastric cancer patients. Jpn J Surg 1983; 13:312–316.
23. Oka M, Mitsunaga H, Hazama S, et al.: Natural killer activity and serum immunosuppressive acidic protein levels in esophageal and gastric cancers. Surg Today 1993;23:669–674.
24. Shibata Y, Tamura K, Ishida N: In vivo analysis of the suppressive effects of immunosuppressive acidic protein, a type of α_1-acid glycoprotein, in connection with its high level in tumor-bearing mice. Cancer Res 1983;43:2889–2896.
25. Sawada M, Okudaira Y, Matsui Y, Shimizu Y: Immunosuppressive acidic protein in patients with ovarian cancer. Cancer 1983; 52:2081–2085.
26. Fujimoto S, Minami T, Ishigami H, et al.: Further studies on immunosuppressive acidic protein (IAP) in gastrointestinal cancer patients—with particular emphasis on relation to immunologic parameters. Jpn J Cancer Clin 1981;27:222–226 (Jpn).
27. Saijo N, Ozaki A, Beppu Y, et al.: Analysis of metastatic spread and growth of tumor cells in mice with depressed natural killer activity by Anti-asialo GM1 antibody or anticancer agents. Cancer Res Clin Oncol 1984;107:157–163.
28. Wiltrout RH, Herberman RB, Zhang S, et al.: Role of organ-associated NK cells in decreased formation of experimental metastases in lung and liver. J Immunol 1985;134:4267–4275.
29. Ishida H, Iwama T, Ide A, et al.: An experimental study on portal infusion of 5-fluorouracil. Jpn J Cancer Chemother 1992; 19:1532–1535 (Jpn).